GENETIC ANALYSIS OF EIGHT-SPORED ASCI PRODUCED BY GENE *E* IN *NEUROSPORA TETRASPERMA*[1]

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UNLIKE the better-known eight-spored species of Neurospora, *N. tetrasperma* usually produces asci with only four ascospores; each ascospore contains nuclei derived from two of the four meiotic products and is usually heterokaryotic for the mating type alleles. DODGE (1939) showed that eight-spored asci also occurred in *N. tetrasperma*, in crosses heterozygous for the pleiotropic gene *E*. Such crosses often showed a high frequency of aborted asci but when fertile yielded varying proportions of both eight-spored and four-spored asci in the same perithecia. Crosses homozygous for *E* were never fertile. The vegetative cycle was also affected, for ascospores containing the *E* allele usually died as germlings, although their survival frequency could be somewhat increased by enriched media.

The cytology of asci heterozygous for *E* has been previously investigated (DODGE, SINGLETON and ROLNICK 1950; SINGLETON, cited by BRAVER 1952), but these authors, as well as HOWE and HAYSMAN (1966), made only limited genetic analyses. Both the interest and the potential usefulness of *E* made it desirable to gain a further understanding of the nature of this allele. The present analysis of asci heterozygous for *E* aims to interpret the various segregation patterns and to correlate these patterns with earlier cytological observations. Our efforts were greatly facilitated by using one of DODGE's *E* strains which was found to have lost much of the lethality described above, such that ascus abortion was markedly reduced, and all eight ascospores from the asci usually survived the germling stage.

MATERIALS AND METHODS

The eight mutants of *N. tetrasperma* used were *act*(113), *al*(102), *col*(105), *col*(118), *col*(120), *E*(121), *me*(123), and *pan*(124). All of these mutants, as well as the culture media used, have been described previously (HOWE and HAYSMAN 1966).

Because the asci remained optimal for dissection for only very short periods, dissections were usually done about 10 days after conidial subculture of a previously established cross. Crosses were maintained at room temperature (23–28°C). All asci were dissected and isolated serially, but without distinction between the apex and base, except where specified. To improve germination the isolated ascospores were aged under conditions of high humidity, so as to avoid drying of the slants, prior to heat activation. Ascospore viability of about 68% was obtained after aging for at least three weeks at room temperature.

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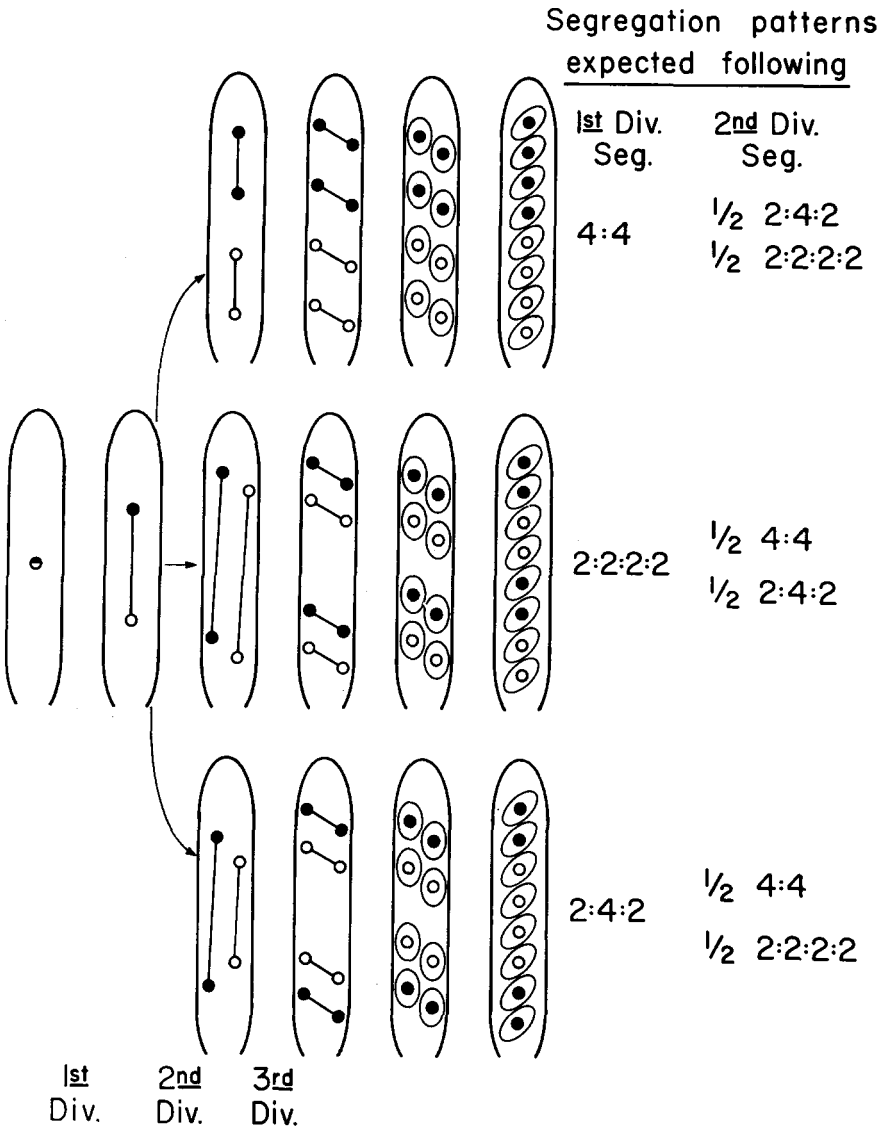

FIGURE 1.—Diagram of the development of eight-spored asci heterozygous for *E* in *N. tetrasperma* (adapted from other authors; see text) to account for the three segregation patterns observed genetically in the present study. To the right are the patterns expected following first division segregation (shown in diagram) and second division segregation of an allelic-pair. *Upper Row, Second Division:* Tandem spindles and no nuclear passing. *Middle Row, Second Division:* Overlapping spindles and passing of a nucleus beyond a nonsister nucleus. *Lower Row, Second Division*: Overlapping spindles and passing of a nucleus beyond two nonsister nuclei.

The *E* allele was scored by crossing each isolate from every ascus to both *EA* and *Ea* tester stocks. All isolates containing the *E* allele were identified by failure to show fertility with either tester, since $E \times E$ crosses are infertile.

Corrected chi-square values were obtained by YATES' correction.

Terminology: The terms concordance and discordance refer to the two members of an ascospore-pair (sister ascospores) being adjacent or nonadjacent, respectively. Asci having the two members of all four ascospore-pairs adjacent are defined as concordant; asci with the two members of at least one ascospore-pair nonadjacent are defined as discordant. Asci are defined as tetratype if at least two segregating allelic-pairs show the tetratype relationship. Ditype asci are defined as those having all segregating allelic-pairs in the ditype relationship. This terminology is used only with eight-spored asci.

RESULTS AND DISCUSSION

Comparison of cytological and genetic studies: The cytology of asci heterozygous for *E* revealed two types of spindle orientations at the second meiotic division (DODGE, SINGLETON and ROLNICK 1950; SINGLETON, cited by BRAVER 1952). In the more frequent type, the two spindles overlap leading to nuclear passing and the consequent location of two nonsister nuclei at each end. In the less frequent type, the two spindles are in tandem resulting in the even spacing of the four nuclei along the longitudinal axis of the ascus and the consequent location of two sister nuclei at each end.

The present genetic study reveals that the mating type alleles, although known to segregate consistently with the centromere at the first meiotic division (HOWE and HAYSMAN 1966), nevertheless show three different segregation patterns, 4:4, 2:2:2:2, and 2:4:2, in serially dissected asci. We propose that these three different segregation patterns of the centromere marker may be reconciled in the following manner with the two different spindle orientations just described. The 4:4 pattern probably arises from the infrequent tandemly oriented second division spindles (Figure 1, upper row). The 2:2:2:2 and 2:4:2 patterns probably arise from the more frequent overlapping second division spindles; each of these latter two patterns, although genetically distinguishable, results from different degrees of nuclear passing (Figure 1, middle and lower rows), which probably would not be cytologically distinguishable, and which were, therefore, reported as only one type of event by SINGLETON. It will be shown that our observed frequencies of segregation patterns are reasonably consistent with the frequencies SINGLETON reported for the two different types of spindle orientations. The segregation patterns expected for markers segregating at the second division, assuming the same cytogenetic mechanisms just proposed, are also presented in Figure 1.

Summation of asci analyzed: A total of 515 asci was dissected and isolated serially from five different crosses heterozygous for *E*. Ten of the 515 asci had allelic ratios other than 1:1 for one or more allelic-pairs and are not considered further; complete genotypes of all 515 asci are given elsewhere (CALHOUN 1968). Ascospore viability in the remaining 505 asci was as follows:

Number of viable ascospores per ascus									Total
0	1	2	3	4	5	6	7	8	asci
77	17	11	17	50	18	42	82	191	505

Our analysis is based upon the 273 asci that contained at least seven viable ascospores, which is the minimum viability needed to attempt an unbiased interpretation of ascospore arrangements. A total of 125 of the 273 asci (45.8%) was concordant; 148 (54.2%), discordant. Results and interpretations for these two groups will be presented separately.

Concordant asci: There were 79 ditypes and 46 tetratypes among the 125 concordant asci. Each of these two ascus types is further grouped according to the segregation patterns shown by the mating type alleles (Table 1). Since mating type segregates consistently at the first division, a second marker showing the same segregation pattern may be assumed to have segregated also at the first

TABLE 1

Genotypes of 125 concordant eight-spored asci serially dissected from five different crosses

Cross	Segregation pattern of mating type alleles	Ascus type*	Number of asci	Genotype of ascospore-pairs			
1. +*Ea* × *al*+*A*	4:4	D	1	+Ea	+Ea	al+A	al+A
	2:2:2:2	D	4	alEA	++a	alEA	++a
		D	1	al+A	+Ea	al+A	+Ea
		T	1	alEA	++a	al+A	+Ea
	2:4:2	D	1	al+A	+Ea	+Ea	al+A
2. ++*Ea* × *act(113)col(118)*+*A* (*act*=m in these 23 asci to avoid confusion with *a* mating type allele)	4:4	D	2	++Ea	++Ea	mc+A	mc+A
		D	1	mc+a	mc+a	++EA	++EA
		D	2	mcEA	mcEA	+++a	+++a
		T	1	mcEA	mc+A	++Ea	+++a
	2:2:2:2	D	3	mc+A	++Ea	mc+A	++Ea
		D	6	mc+a	++EA	mc+a	++EA
		D	1	+++A	mcEa	+++A	mcEa
		T	1	+++a	mcEA	++Ea	mc+A
		T	1	mc+a	++EA	mcEa	+++A
		T	1	mc+a	+cEA	+++a	m+EA
		T	1	+++A	++Ea	mc+A	mcEa
		T	1	mc+A	mcEa	+++A	++Ea
	2:4:2	D	1	++Ea	mc+A	mc+A	++Ea
		D	1	+++a	mcEA	mcEA	+++a
3. +*pan EA* × *col(120)*++*a*	4:4	D	2	+pEA	+pEA	c++a	c++a
		D	1	c+EA	c+EA	+p+a	+p+a
		D	3	c++A	c++A	+pEa	+pEa
		D	1	c+Ea	c+Ea	+p+A	+p+A
		T	1	cp+a	+p+a	++EA	c+EA
		T	1	+p+a	cp+a	c+EA	++EA
		T	1	+pEA	c+EA	+++a	cp+a
	2:2:2:2	D	7	c++A	+pEa	c++A	+pEa
		D	4	+pEA	c++a	+pEA	c++a
		D	4	+p+A	c+Ea	+p+A	c+Ea
		D	4	+p+a	c+EA	+p+a	c+EA
		D	1	cpEA	+++a	cpEA	+++a
		T	1	++EA	cp+a	c+EA	+p+a

TABLE 1—Continued

Genotypes of 125 concordant eight-spored asci serially dissected from five different crosses

Cross	Segregation pattern of mating type alleles	Ascus type*	Number of asci	Genotype of ascospore-pairs			
		T	1	+pEA	+++a	cpEA	c++a
		T	1	+++A	cpEa	c++A	+pEa
		T	1	+p+A	c+Ea	cp+A	++Ea
		T	1	+pEA	c++a	cpEA	+++a
		T	1	+p+a	c+EA	c++a	+pEA
4. *+pan EA × col(105)++a*	4:4	D	1	c++a	c++a	+pEA	+pEA
		D	2	+pEa	+pEa	c++A	c++A
		D	1	c+Ea	c+Ea	+p+A	+p+A
		T	1	+++A	c++A	cpEa	+pEa
		T	1	c++a	+++a	+pEA	cpEA
		T	1	+++a	c++a	+pEA	cpEA
		T	1	+p+a	cp+a	c+EA	++EA
		T	1	cpEa	+pEa	+++A	c++A
		T	2	++Ea	c+Ea	cp+A	+p+A
	2:2:2:2	D	3	c++A	+pEa	c++A	+pEa
		D	5	+p+a	c+EA	+p+a	c+EA
		D	2	c++a	+pEA	c++a	+pEA
		D	5	+p+A	c+Ea	+p+A	c+Ea
		D	1	cpEa	+++A	cpEa	+++A
		T	2	c+EA	cp+a	++EA	+p+a
		T	2	c++a	cpEA	+++a	+pEA
		T	1	cpEa	c++A	+pEa	+++A
		T	1	+p+A	cpEa	c++A	++Ea
		T	1	+p+A	c+Ea	cp+A	++Ea
		T	2	+pEA	c++a	cpEA	+++a
		T	1	cpEa	+++A	+pEa	c++A
		T	1	c++A	cpEa	+++A	+pEa
		T	1	cp+A	c+Ea	+p+A	++Ea
	2:4:2	D	1	c++A	+pEa	+pEa	c++A
		T	1	+p+A	c+Ea	++Ea	cp+A
		T	1	c++A	+p+a	+pEa	c+EA
		T	1	cp+a	c+EA	++EA	+p+a
5. *+Ea × me+A*	4:4	D	2	mEA	mEA	++a	++a
		D	1	++A	++A	mEa	mEa
	2:2:2:2	D	2	m+A	+Ea	m+A	+Ea
		D	1	m+a	+EA	m+a	+EA
		D	1	++A	mEa	++A	mEa
		T	1	++a	mEA	m+a	+EA
		T	3	m+a	mEA	++a	+EA
		T	1	mEa	++A	+Ea	m+A
		T	1	m+a	+EA	++a	mEA
		T	1	mEa	m+A	+Ea	++A
	2:4:2	T	1	mEa	++A	m+A	+Ea

* D signifies ditype with respect to all segregating pairs of alleles; T signifies tetratype with respect to at least two segregating pairs of alleles.

TABLE 2

Frequencies of the three segregation patterns for the mating type alleles in 125 concordant ditype (D) and tetratype (T) asci

Segregation pattern	Cross and ascus type										Total asci		
	1		2		3		4		5				
	D	T	D	T	D	T	D	T	D	T	D	T	Percent
4:4	1	0	5	1	7	3	4	7	3	0	20	11	24.8
2:2:2:2	5	1	10	5	20	6	16	12	4	7	55	31	68.8
2:4:2	1	0	2	0	0	0	1	3	0	1	4	4	6.4
χ^2_1*	0.38		1.17		0.18		2.22		1.76		0.07		

* Chi-square tests show that segregation patterns are independent of ascus types.

division; a second marker showing a different segregation pattern than mating type may be assumed to have segregated at the second division. It may be seen from the groupings in Table 1 that three different segregation patterns for mating type occurred and that the other markers in any given ascus sometimes showed the same pattern as mating type and sometimes did not. Consequently, all three patterns occurred following first division segregation, and all three occurred following second division segregation. Centromere markers have been used similarly in other studies to classify segregations of markers in asci having uncertain ascospore arrangements (HAWTHORNE and MORTIMER 1960; BERG 1966).

In Table 2, chi-square values from contingency tables are shown which indicate independence of segregation patterns and ascus types (ditypes and tetratypes). That is, none of the three segregation patterns was any more likely to occur in one ascus type than in the other. Consequently, discordance did not occur in significant frequencies at the ends of the 4:4 ditype asci nor at the center of the 2:4:2 ditype asci; thus discordant ditype asci were not misclassified as concordant asci with significant frequency. Such misclassification would have increased the frequencies of the 4:4 and 2:4:2 patterns relative to the 2:2:2:2 pattern in ditype asci.

The associations between the segregation patterns for mating type and for each of the other eight loci involved in the five crosses studied are shown in Table 3. The data are consistent with random disjunction at the second meiotic division. The percentages of second division segregation are also shown in Table 3, from which the centromere distance for each of the eight loci may be obtained by dividing by two.

The relationship of the ascospores to the apex and base of the ascus was recorded for crosses 1 and 3. The 4:4 and 2:2:2:2 first division segregation patterns for an allele at four different loci segregating in these two crosses, and representing three different linkage groups, are given in Table 4. The apex to base ratios for each pattern do not differ significantly from a 1:1 chance expectation, based on corrected chi-square values. Thus, the data are consistent with random disjunction at the first meiotic division.

TABLE 3

Association of the segregation patterns for the mating type alleles with the segregation patterns for the alleles at eight other loci segregating in 125 concordant asci

Locus and linkage group	Cross	Segregation pattern for the mating type alleles: 4:4(31 asci) Segregation pattern for the alleles at eight other loci: 4:4 Number of asci	2:4:2 Number of asci	2:2:2:2 Number of asci	Segregation pattern for the mating type alleles: 2:2:2:2(86 asci) Segregation pattern for the alleles at eight other loci: 2:2:2:2 Number of asci	4:4 Number of asci	2:4:2 Number of asci	Segregation pattern for the mating type alleles: 2:4:2(8 asci) Segregation pattern for the alleles at eight other loci: 2:4:2 Number of asci	4:4 Number of asci	2:2:2:2 Number of asci	Percentage* 2nd division segregation
al(102), I	1	1	0	0	6	0	0	1	0	0	0.0
pan(124), IV	3	9	1	0	25	0	1	0	0	0	3.8
	4	11	0	0	27	1	0	4	0	0	. .
E(121), VI	1	1	0	0	5	0	1	1	0	0	4.0
	2	5	0	1	13	0	2	2	0	0	. .
	3	10	0	0	26	0	0	0	0	0	. .
	4	11	0	0	28	0	0	3	1	0	. .
	5	3	0	0	11	0	0	1	0	0	. .
act(113), V	2	6	0	0	12	2	1	2	0	0	13.0
col(118), V	2	6	0	0	12	3	0	2	0	0	13.0
col(120), IV	3	7	2	1	20	1	5	0	0	0	25.0
col(105), IV	4	4	6	1	16	7	5	2	1	1	48.8
me(123), VI	5	3	0	0	4	4	3	0	0	1	53.3

* Percentage of asci having segregation patterns different than those for mating type.

Discordant asci: There were 84 ditypes and 64 tetratypes among the 148 discordant asci. Positions of discordance (Table 5) occurred as follows: at one end of the ascus, 95 (64.2%); at the center, 17 (11.5%); at both ends, 16 (10.8%); at one end and the center, 15 (10.1%); and at both ends and the center, 5 (3.4%).

In the group having discordance at one end, 44 asci were tetratype, whereas in

TABLE 4

Numbers of asci showing various positions of alleles with respect to the apex and base of the ascus for markers segregating at the first division in the concordant asci from crosses 1 and 3

Allele	Cross	First division segregation pattern 4:4 Position of allele in the ascus: Apex	Base	First division segregation pattern 2:2:2:2 Position of allele in the ascus: Apex	Base	Apex to base ratio
A	1	0	1	6	0	28:15
	3	6	4	16	10	
E(121)	1	1	0	4	1	22:20
	3	6	4	11	15	
col(120)	3	4	3	9	11	13:14
pan(124)	3	5	4	15	10	20:14

TABLE 5

Segregation patterns for mating type initially found in 148 discordant eight-spored asci serially dissected from five different crosses, and reclassification of the 57 least ambiguous discordances to give the most probably correct segregation patterns. D = ditype; T = tetratype. m = A or a; + = A or a mating type alleles

Location of discordance	Segregation pattern for mating type initially found in D & T asci†		Number of asci in the five crosses 1	2	3	4	5	Total asci	Reclassified segregation pattern for mating type
One end	$\underline{mmmm}\underline{+}\underline{+}++$	D‡	.	.	.	.	.	.	*
		T	0	0	0	3	1	4	4:4
	$\underline{m}+\underline{m}+mm++$	D	2	7	3	11	1	24	*
		T	0	1	0	10	7	18	2:2:2:2
	$\underline{m}+\underline{m}+++mm$	D	3	2	1	3	1	10	*
		T	0	0	1	0	2	3	2:4:2
	$\underline{m}+\underline{+}\underline{m}++mm$	D	1	5	5	1	5	17	*
		T	0	2	1	11	5	19	2:2:2:2 or 2:4:2
Center	$\underline{mmm}+\underline{m}+++$	D^s	0	0	1	1	0	2	*
		T	0	0	0	0	0	0	4:4
	$\underline{mm}+\underline{m}+m++$	D	2	0	2	2	1	7	*
		T	0	0	2	2	1	5	2:2:2:2
	$\underline{mmm}+\underline{+}m++$	D	0	0	1	0	0	1	*
		T	0	0	0	1	0	1	4:4 or 2:2:2:2
	$\underline{mm}+\underline{+}+\underline{+}+mm$	D‡	.	.	.	.	.	.	*
		T	0	0	0	1	0	1	2:4:2
Both ends	$\underline{m}+\underline{m}+m+m+$	D	0	0	0	1	0	1	*
		T	0	1	0	0	1	2	2:2:2:2
	$\underline{m}+\underline{m}+\underline{+}+m+m$	D	0	1	0	0	0	1	*
		T	0	0	0	0	0	0	2:4:2
	$\underline{m}+\underline{+}\underline{mm}++m$	D	0	0	0	2	0	2	*
		T	0	0	0	0	2	2	2:2:2:2 or 2:4:2
	$\underline{m}+\underline{m}+\underline{+}mm+$	D	0	2	2	1	1	6	*
		T	0	0	0	1	1	2	2:2:2:2 or 2:4:2
One end and center	$\underline{mm}+\underline{m}+\underline{+}m+$	D^s	2	2	1	2	1	8	*
		T	0	0	0	1	0	1	
	$\underline{mmm}+\underline{+}\underline{+}m+$	D	0	0	0	1	0	1	*
		T	0	0	0	0	0	0	
	$\underline{mm}+\underline{m}+\underline{+}++m$	D	0	0	0	0	0	0	*
		T	0	0	0	2	1	3	
	$\underline{mm}+\underline{m}+\underline{+}\underline{m}+$	D^s	.	.	.	.	.	.	*
		T	0	0	0	1	0	1	
	$\underline{mmm}+\underline{+}\underline{+}++m$	D	0	0	1	0	0	1	*
		T	0	0	0	0	0	0	
Both ends and center	$\underline{mmm}+\underline{m}+\underline{+}++$	D^s	.	.	.	.	.	.	*
		T	0	0	0	1	0	1	
	$\underline{m}+\underline{mm}+\underline{+}m+$	D	0	0	1	0	0	1	*
		T	0	0	0	0	0	0	
	$\underline{m}+\underline{+}+\underline{mm}+m$	D	0	1	0	0	0	1	*
		T	0	0	0	1	0	1	
	$\underline{m}+\underline{+}+\underline{mmm}+$	D	0	0	0	1	0	1	*
		T	0	0	0	0	0	0	
Total asci			10	24	22	61	31	148	

* Not reclassified because of ambiguity.

† Type underlined indicates members of a spore-pair (applies only to tetratypes, where spore-pairs are distinguishable).

‡ Scored as concordant 4:4 and 2:4:2, respectively, first division segregation patterns.

^{s} Indistinguishable.

the group having discordance at the center, seven were tetratype. This 44:7 ratio for end and center is significantly different from a 2:1 chance expectation at the 1% level (corrected chi-square = 7.96, 1 df). The total tetratype data for end and center in Table 5 are also significant at the 1% level. Thus, discordance occurred preferentially at an ascus end.

The position of discordance in relation to the apex and base was known in 26 asci with discordances at one end, from crosses 1, 3, and 5. The 17:9 apex:base ratio found is not significantly different from a 1:1 chance expectation (corrected chi-square = 1.88, 1 df). Thus, no preference was shown for one end of the ascus over the other in relation to end discordances.

The frequency of discordant asci was too high to be attributed to dissecting errors. We postulate that the discordance resulted primarily from nuclear passing at the third division and/or from ascospore slippage when the ascospores moved from the biseriate to the uniseriate position (Figure 1). The intermittent occurrence of nuclear passing at the third division should not be unexpected in view of the fact that nuclear passing at both the second and third divisions occurs regularly in the wild type ascus of this species.

The 57 tetratype asci with discordance at one end, or at the center, or at both ends were reclassified by assuming that these discordances were the direct result of nuclear passing at the third division and/or of ascospore slippage; the reclassified ascospore patterns obtained by making the simplest rearrangements under this assumption are shown, with respect to the mating type alleles, in Table 5. Reclassification was not done for any of the 84 ditypes, nor for the remaining seven tetratypes having discordances at one end and the center or at both ends and the center because of the ambiguity encountered. After reclassification, 4 or 5 of the 57 tetratype asci had the 4:4 segregation pattern for the mating type alleles, and 52 or 53 had the 2:2:2:2 or 2:4:2 patterns. It will be recalled (Table 2) that in the concordant tetratype asci there were eleven 4:4 segregation patterns for the mating type alleles and thirty-five 2:2:2:2 or 2:4:2 patterns. A contingency table used to test for independence of first division segregation patterns and ascus types (reclassified tetratypes and concordant tetratypes) showed a significant chi-square value (4.3, 1 df) at the 5% level. Thus discordance occurred significantly less frequently in asci showing the 4:4 pattern for the mating type alleles than in asci showing the other two patterns.

It seems likely that the probability of discordance would be a function of the proximity of the nuclei during ascus development. Thus, relatively little discordance was found in the 4:4 first division segregation pattern believed to result from the even dispersal of the four telophase nuclei at the second division (Figure 1). On the other hand, the 2:2:2:2 and 2:4:2 patterns showed discordances frequently at the ends of the asci, where nuclei are closely associated (Figure 1), but less frequently at the center, where nuclei are not so close together.

Genetic estimates of spindle behavior frequencies: In order to base such estimates upon both concordant and discordant asci, only tetratypes were used, because of the difficulty of identifying sister ascospores in discordant ditypes. There

were 46 concordant tetratypes (Table 2) and 57 reclassifiable discordant tetratypes, 33 of which were reclassified into the most probably correct segregation pattern and 24 of which could only be reclassified as either of two most probably correct patterns (Table 5). The frequency of the 4:4 pattern could be determined rather precisely, since either 15 or 16 of the 103 tetratypes under consideration (14.6% or 15.5%) showed this pattern. The frequencies of the other two patterns could not be precisely determined because of the dichotomy in reclassification of 24 of the asci; these frequencies are therefore stated only as ranges, namely, 54.4% to 77.7% for the 2:2:2:2 pattern and 7.8% to 30.1% for the 2:4:2 pattern, depending upon the pattern to which the 24 asci are assigned.

The values 14.6% or 15.5% for the 4:4 pattern represent our genetic estimate of the frequency of tandemly oriented second division spindles, which we have postulated give rise to the 4:4 pattern; these values are in reasonably good agreement with SINGLETON's cytological determination of "about 10%" tandemly oriented spindles (BRAVER 1952). Overlapping spindles apparently occurred much more frequently in ascus development, during which the less extreme type of nuclear passing predominated (54.4% to 77.7%); this type of passing is believed to result in the 2:2:2:2 pattern (Figure 1, middle row).

Four-spored asci: Crosses heterozygous for *E* (121) produce some four-spored asci in the same perithecia in which the generally more numerous eight-spored asci are produced. 54 and 49 four-spored asci, respectively, were analyzed from crosses 4 and 5, both of which were heterozygous for *E*. These asci showed the same genetic characteristics recently found to be typical of four-spored asci from crosses in which *E* is not segregating (CALHOUN and HOWE 1968). The four-spored asci from both of these origins, as well as eight-spored asci, have all given consistently similar centromere distances. Thus, four-spored asci have not been shown to be detectably different whether originating from crosses in which *E* is segregating or from crosses lacking *E*. The basis for this apparently frequent variation in expression of the *E* allele is not yet clear.

Significance of gene E: The major significance of gene *E* probably lies in its pronounced effect upon meiosis and certain other events during ascus development. The dimensions of the ascus become modified. Nuclear passing at the third division, and sometimes at the second division, is apparently eliminated, and the nuclei are subsequently delimited singly instead of pair-wise during formation of the ascospore walls. The homokaryotic ascospores thereby produced have the practical advantage of being much more amenable to genetic analysis than are the heterokaryotic ascospores of the four-spored ascus.

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SUMMARY

Crosses heterozygous for the gene *E* produce eight-spored asci having two especially noteworthy characteristics: (1) both first and second division segre-

gations are manifested by all three segregation patterns, 4:4, 2:2:2:2 and 2:4:2 and (2) about half of the asci are discordant, i.e., the two members of at least one ascospore-pair (sister ascospores) are nonadjacent. The 4:4 pattern arises from tandemly oriented spindles at the second meiotic division which have a genetically determined frequency of about 15%. Much more commonly, however, spindles overlap at the second division and give rise to the two types of nuclear passing that produce the 2:4:2 and 2:2:2:2 patterns, the latter more frequently. Discordance probably results from nuclear passing occurring intermittently at the third division and/or from ascospore slippage. Because of the reliability of the mating type locus as a centromere marker, discordant tetratype asci may usually be reclassified to fit the most probably correct ascospore arrangement. The four-spored asci which are usually also present in crosses heterozygous for *E* do not differ detectably in genetic behavior from four-spored asci from crosses in which *E* is not segregating.

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